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A novel selective γ-aminobutyric acid transport inhibitor demonstrates a functional role for GABA transporter subtype GAT2/BGT-1 in the CNS

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Abstract

The system of GABA transporters in neural cells constitutes an efficient mechanism for terminating inhibitory GABAergic neurotransmission. This transport system is an important therapeutical target in epileptic disorders, but potentially also in other neurological disorders. Thus, selective intervention in GABA uptake has been the subject of extensive research for several decades. In a series of lipophilic diaromatic derivatives of (*RS*)-3-hydroxy-4-amino-4,5,6,7-tetrahydro-1,2-benzisoxazole (*exo*-THPO), *N*-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-3-hydroxy-4-(methylamino)-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol (EF1502) turned out to be an equipotent inhibitor at the mouse transporters GAT1 and GAT2 (BGT-1) but inactive at GAT3 and GAT4. This novel pharmacological profile among GABA uptake inhibitors prompted a thorough investigation of the in vivo properties of this compound. These investigations have for the first time demonstrated a functional role for GABA transporter subtype GAT2/BGT-1, which points to the therapeutic relevance of inhibiting this transporter subtype. An overview of the development and characterisation of EF1502 is presented here.

Keywords: GABA; GABA uptake; GAT1; mGAT2; BGT-1; Epilepsy; Anti-convulsive; GABA transporter; EF1502

1. Introduction

A number of central nervous system disorders have been linked to hypoactivity in inhibitory neurotransmission elicited by γ-aminobutyric acid (GABA) (Lloyd and Morselli, 1987). In addition to the treatment of anxiety and sleep disorders, drugs that enhance GABA-mediated inhibition have been found highly effective in the management of seizure disorders. In this respect, the clinical effect of allosteric $GABA_A$ receptor modulators such as the benzodiazepines (see Costa et al., 1975 and Haefely, 1978 for review), the barbiturates (Ransom and Barker, 1976) and the irreversible GABA transaminase (GABA-T) inhibitor vigabatrin (Lippert et al., 1977) is linked to their ability to enhance GABA-mediated inhibitory neurotransmission.

Additionally, inhibition of GABA transport has gained much attention as an anticonvulsive strategy. Since GABAergic neurotransmission is terminated by uptake into the neuron or surrounding glia cells, inhibition of GABA transporters responsible for uptake would prolong the GABAergic signal in a use-dependent manner, thereby counteracting GABA hypoactivity (Schousboe et al., 2004). Although many GABA uptake inhibitors possess antiepileptic properties only one compound (i.e. Tiagabine) with this mechanism has been approved so far for the treatment of epileptic disorders (see Suzdak and Jansen, 1995 for review and references).

The concept of a pharmacological distinction between neuronal and astroglial GABA transport evolved early on from studies of the inhibitory action of compounds such as β-alanine, 2,4-diaminobutyric acid (DABA), and *cis*-3-aminocyclohexane carboxylic acid (Iversen and Kelly, 1975; Neal and Bowery, 1977) followed by other GABA analogues such as β-proline and 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol (THPO)

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(Schousboe et al., 1978, 1981). It was speculated that since glial GABA uptake leads to degradation and hence a loss from the neurotransmitter pool as opposed to neuronal uptake which allows reutilization as a neurotransmitter, preferential inhibition of astrocytic GABA transport inhibition could be advantageous with regard to seizure control (Schousboe et al., 1983). In keeping with this hypothesis, the neuronal GABA transport inhibitor DABA was shown to act as a proconvulsant (Meldrum et al., 1982). The proconvulsant action associated with neuron specific GABA transport inhibitors is presumed to result from a depletion of synaptic pools of GABA (Schousboe et al., 1983).

To date, five different high affinity GABA transporters have been cloned of which VGAT is a vesicular transporter and GAT1–GAT4 belong to the large family of 12-transmembrane spanning Na^+/Cl^- coupled neurotransmitter transporters (Miller et al., 1997; Schousboe and Kanner, 2002). The nomenclature GAT1–GAT4 is used for the mouse transporters; whereas, the corresponding transporters in rats and humans are named GAT-1, BGT-1, GAT-2 and GAT-3, respectively. In the present review the ''mouse nomenclature'' GAT1–GAT4 will be used.

While the role of GAT1 has been extensively investigated due to the availability of highly GAT1-selective inhibitors, the role of the other subtypes is less well described due to the lack of highly specific inhibitors. Recently, the synthesis and characterization of a GAT1/GAT2 (BGT-1)-selective inhibitor *N*-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-3-hydroxy-4-(methylamino)-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol (EF1502) was described. The availability of this compound has, for the first time, demonstrated a functional role for GABA transporter subtype GAT2 (Clausen et al., 2005 and White et al., 2005). The development of this interesting inhibitor, as well as its synthesis and pharmacological characterization will be reviewed.

2. Development of selective GABA uptake inhibitors using muscimol as lead structure

Muscimol (Fig. 1) is a naturally occurring GABA analogue containing the acidic 3-isoxazolol heterocycle, which acts as a bioisosteric substitute for the carboxylic acid and induces conformational restrictions on the carbon backbone of GABA. This leads to pharmacological restrictions and muscimol interacts with GABA receptors and transporters, as well as GABA-T (Krogsgaard-Larsen et al., 1975). The receptor and transporter activity was separated by very simple structural transformations of muscimol. Thus, conformational restriction of muscimol by an ethylene linker gave 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP) (Krogsgaard-Larsen et al., 1977, 1983, 2000), which selectively activates receptors without any apparent effects on GABA transport (Table 1). THIP does not display the high affinity of muscimol in a synaptosomal GABA binding assay, but is currently known as a potent super-agonist at δ-subunit containing GABA receptors (Adkins et al., 2001). Moving the basic nitrogen in the piperidine ring led to the β-alanine analogue THPO (Krogsgaard-Larsen and Johnston, 1975; Schousboe et al., 1981), which displayed essentially no receptor activity but improved uptake inhibitory properties. This facilitated the discovery of two very selective and potent transport inhibitors, nipecotic acid and guvacine (Krogsgaard-Larsen et al., 1975), which are the cyclic amino acid parents of THPO. These two naturally occurring compounds were completely devoid of measurable receptor activity and therefore highly selective GABA transport inhibitors (Table 2).

Another important transformation in the development of selective GABA uptake inhibitors was the addition of the lipophilic aromatic side chain 4,4-diphenylbut-3-en-1-yl (DPB) to nipecotic acid and guvacine as in *N*-DPB-nipecotic acid

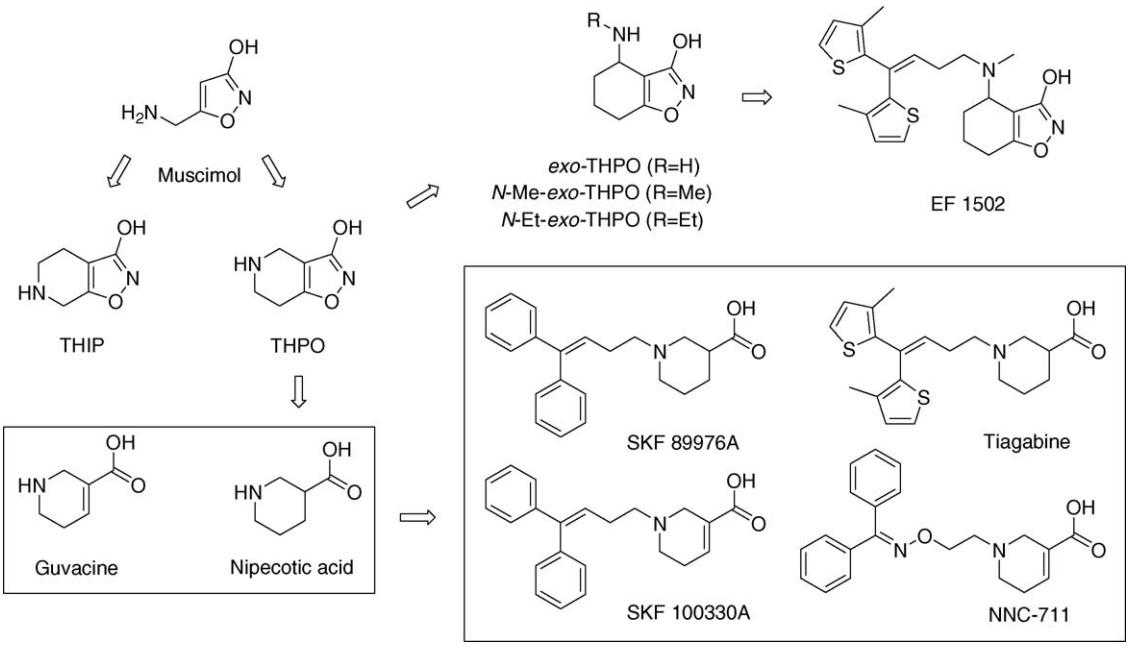

Fig. 1. Development of selective GABA uptake inhibitors from muscimol.